bio-protocol Preprint

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ChIP-seq

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An abbreviated version of this protocol was published in eLIFE in Dec 2018

Role of the pre-initiation complex in Mediator recruitment and dynamics

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Detailed protocol

Current Morse Lab ChIP Protocol

Day 1

- Grow 50mL culture to doubling phase, with OD_{600} of 0.6 to 1.0
- When cells are ready to collect, add 1.5mL of 37% formaldehyde and crosslink for 15min. Mix occasionally, keeping at appropriate temperature (especially if doing experiments with *ts* mutants). Usually we shake at low speed (60 rpm or so) in the appropriate shaking incubator.
- Add 2.5mL of 2.5M glycine for 5min to stop the crosslinking and mix occasionally.
- Transfer to 50mL tubes and spin down pellet at 4°C for 5min at 2000rpm. Discard supernatant in collection bottle in fume hood (because of formaldehyde).
- Wash pellet twice by resuspending in 20mL cold 1xTBS and spinning at 4° for 5min at 2000rpm.
- Resuspend pellet in ~600-700µL of FA Lysis Buffer with 0.1% SDS and Protease Inhibitors (PI) (PI per 10mL Buffer: 5µL pepstatin [2mg/mL] and 10µL leupeptin [1mg/mL]). It may be easiest to make up 2 ml or more (depending on number of ChIP samples) in a plastic tube and aliquot from there.
- Transfer to microcentrifuge tube filled to about 500µL with glass beads. Place tube in automatic vortexer in cold room for 40min. While cells are being lysed, turn on the recirculating water bath that is used with the Diagenode Bioruptor (sonicator) to allow it to cool. Put your name on the sign up sheet on the door.
- Puncture bottom of tube with hot dissecting needle and place over empty microcentrifuge tube. Spin at 8000rpm for 15-20sec. Resuspend pellet with the supernatant liquid and transfer entire contents to 15mL BD Falcon tube. Do not use the semi-opaque Falcon tubes.
- Sonicate samples on high in Diagenode Bioruptor for 15min (30 sec on, 30 sec off) in cold, recirculating waterbath to shear DNA into fragments of 500bp or smaller. Be sure to turn off the pump and recirculating water bath when you are done.
- Transfer everything to microfuge tube and spin at 4° for 15min at 14,000rpm to pellet debris.
- Transfer supernatant to clean microfuge tube and spin at 4° for 30min at 14,000rpm to clear chromatin.
- Transfer supernatant to new microfuge tube. This is your whole cell extract (WCE). Store at -80°.
- If proceeding, incubate 180µL of WCE with 2-10ug of antibody and bring the total volume up to 500µL with FA Lysis Buffer with SDS & PI. (For ChIP-seq we use the entire WCE, ~700 µl, after removing 36 ul for input.) At this point, a separate lysis buffer with protease inhibitors WITHOUT SDS can be used if there is concern for interaction of the protein and the SDS. Incubate with gentle agitation at 4° overnight.

Day 2

- Prepare beads (protein A or G, Amersham/GE; no blocking necessary) by resuspending them in their slurry. Beads should be aliquotted from the original container to avoid too many users going into the main stock. If the volume of liquid above the settled beads is significantly less than the volume of the settled beads, add 20% ethanol to bring up the volume. Wash 30µL of beads per IP sample twice with 850µL FA Lysis Buffer with PI, spinning for 1m at 4,000rpm at room temp. Resuspend in fresh FA Lysis Buffer equal to original volume. Alternatively, beads sufficient to process up to four IP samples (120 ul) can be washed in one batch using about 850 ul FA Lysis Buffer. If this is done, we recommend using a bit extra to facilitate removal of 30 ul aliquots in the next step without excessive frothing due to the detergent present in the FA Lysis Buffer.
- Aliquot 30µL of beads into each IP sample. Alternatively, IP samples can be spun 5 min at 4˚C at 13,000 rpm, and the supernatant added to microfuge tubes each containing 30 ul of the washed beads. Return samples to gentle agitation at 4° for 90 min.
- Transfer IP samples with beads to Spin-X column and spin at 4,000 rpm for 1min (room temp, with lids closed) to remove supernatant. Lift out Spin-X column from reservoir tube, dump flow-through, and replace Spin-X column.
- Wash beads with 700µL of FA Lysis Buffer. Rotate for 3min at room temp. Spin for 1min at 4,000rpm. Discard flow-through.
- Wash beads with 750µL of FA Lysis Buffer for 3min with rotation at room temp. Spin for 1min at 4,000rpm and discard flow through.
- Wash beads again with FA Lysis Buffer- High Salt: Rotate for 3min at room temp. Spin for 1min at 4,000rpm. Discard flow-through.
- Wash beads with LiCl Wash Solution: Rotate for 3min at room temp. Spin for 1min at 4,000rpm. Discard flow-through.
- Wash beads with TE: Rotate for 3min at room temp. Spin for 1min at 4,000rpm. Discard flow-through.
- After discarding TE flow through, resuspend beads with 100µL Elution Buffer. Incubate at 70° for 10 min with vigorous shaking (1200 rpm) to elute samples in Eppendorf Thermomixer.
- While IP samples are incubating, prepare input (IT) samples by adding 36ul of WCE to 64ul 1% SDS in TE

- While IP samples are incubating, prepare Input (IT) samples by adding 36µL of WCE to 64µL 1%SDS in TE.
- Remove IP sample from heat and spin columns at 4,000rpm for 1min. Discard columns and beads.
- Incubate all samples, IT and IP, at 100° for 10min with vigorous shaking (1200rpm) to reverse crosslink in Eppendorf Thermomixer.
- Clean samples with QIAGEN PCR Purification protocol. Add 500µL of buffer PB to samples and transfer to QIAcolumn. Spin for 1min at 13,000rpm to bind sample and discard flow through. Wash sample with 750µL of buffer PE. Spin for 1 min at 13,000rpm and discard flow through. Spin column for 1 min at 13,000rpm to dry. Transfer column to new microfuge tube. Add 50µL TE to IP samples and 100µL TE to IT samples for elution. Allow to sit at room temp for 1min to increase recovery. Spin for 1min at 13,000rpm and keep flow through.
- Test samples by Real Time PCR. Use 0.5µL of concentrated IP and 0.5µL of 1:100 IT.
- Store samples at -20°

Solutions

TBS
- 20mM Tris-HCl, pH 8.0
- 150mM NaCl

FA Lysis Buffer
- 50mM HEPES, pH 8.0
- 140mM NaCl
- 1mM EDTA,
- 1% Triton-X
- 0.1% Sodium Deoxycholate

FA Lysis Buffer- High Salt
- 50mM HEPES, pH 8.0
- 500mM NaCl
- 1mM EDTA
- 1% Triton-X
- 0.1% Sodium Deoxycholate

LiCl Wash Buffer
- 10mM Tris-HCl, pH 8.0
- 250mM LiCl
- 0.5% NP-40
- 1mM EDTA
- 0.5% Sodium Deoxycholate

TE Buffer
- 10mM Tris-HCl, pH 8.0
- 1mM EDTA

ChIP Elution Buffer
- 50mM Tris-HCl, pH 7.5
- 10mM EDTA
- 1% SDS

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Morse, R. (2020). ChIP-seq. Bio-protocol Preprint. bio-protocol.org/prep679.
2. Knoll, E. R., Zhu, Z. I., Sarkar, D., Landsman, D. and Morse, R. H.(2018). Role of the pre-initiation complex in Mediator recruitment and dynamics. eLIFE. DOI: 10.7554/eLife.39633